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Potential antimicrobial agents from triazole-functionalized 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-ones

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ABSTRACT

A series of substituted triazole functionalized 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-ones were synthesized by employing click chemistry and further characterized based on ^{1}H NMR, ^{13}C NMR, IR and mass spectral studies. All the synthesized derivatives were screened for their *in vitro* antimicrobial activities. Further, molecular docking studies were accomplished to explore the binding interactions between 1,2,3-triazol-4-yl-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one and the active site of *Staphylococcus aureus* (CrtM) dehydrosqualene synthase (PDB ID: 2ZCS). These docking studies revealed that the synthesized derivatives showed high binding energies and strong H-bond interactions with the dehydrosqualene synthase validating the observed antimicrobial activity data. Based on antimicrobial activity and docking studies, the compounds **9c**, **9d** and **9e** were identified as promising antimicrobial leads.

Antibiotics were claimed as wonder drugs to cure various diseases and have saved millions of lives globally which has considerably improved the public health. Nevertheless, a serious drug-resistance crisis has evolved in the recent years due to overuse and abuse of antibiotics, as well as the lack of research and development towards identifying new drugs by the pharma industry due to increased cost for drug discovery and reduced economic incentives and challenging regulatory requirements.[1,2] The emergence of bacterial resistance pattern towards various antimicrobial agents has generated various drug-resistant strains including methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *S. epidermidis* (MRSE), penicillin-resistant *Streptococcus pneumonia* (PRSP) and vancomycin-resistant enterococci (VRE) which has resulted in increased morbidity and mortality rates and this has mandated a pressing concern for human health.[3–6] Considering these facts, there is an urgent need to identify new types of antibacterial drugs, and/or to search new targets or mechanisms to understand the antimicrobial effects.[7–14] In this regard, attempts were made to target the golden carotenoid pigment, staphyloxanthin produced by *S. aureus*,[15] which functions as a virulence factor for *S. aureus*. The staphyloxanthin pigment acts as an antioxidant and protects the microbial cells against oxidative stress due to host immune defense by reactive oxygen species and neutrophils.[16,17]

The chemistry of heterocyclic compounds continues to be an interesting field to explore for various biological activities. A large volume of research has been carried out on 2*H*-1,4-benzoxazin-3-(4*H*)-ones from a pharmaceutical chemistry perspective. The 2*H*-1,4-benzoxazin-3-(4*H*)-ones and 3,4-dihydro-2*H*-1,4-benzoxazines are considered as privileged scaffolds for the design of biologically active compounds. As shown in Fig. 1, the benzoxazinone-based compounds form an important class of benzo-fused heterocycles exhibiting a wide spectrum of biological activities including antitumor (**I**),[18] antiphlogistic, antipyretic and analgesic effects of 4-aminoalkyl-2,3-dihydro-1,4-benzoxazin-3-ones (**II**) which has led to the proposed use of some of these compounds as efficient pharmaceuticals[19] and indeed, calcium channel blockers based on substituted 1,4-benzoxazinones (**III**)[20] were claimed for their functional role as anti-hypertensives, vasodilators, and anti-ischemic agents. In addition, several 1,4-benzoxazinone derivatives find use as antifungal drugs (**IV**, **V**)[21,22] and broncholytics (**VI**).[23]

(1,2,3)-Triazole moieties are attractive bridging units in view of their stability to metabolic degradation and are capable of hydrogen bonding, which is a favourable aspect for binding the biomolecular targets and can also improve the solubility.[24] Like azoles, triazoles find use in many antifungal drugs and fungicides;

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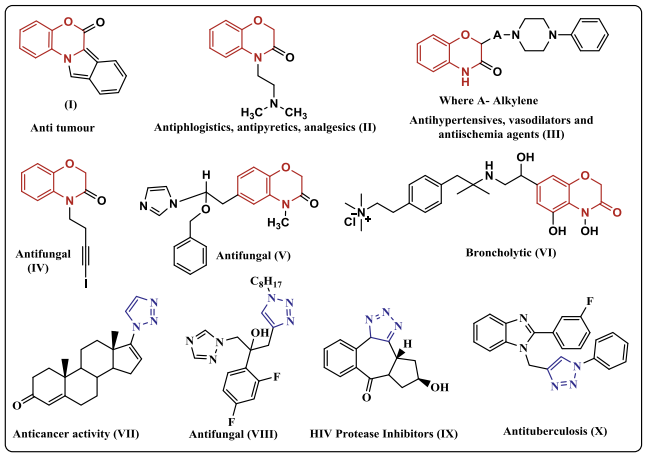
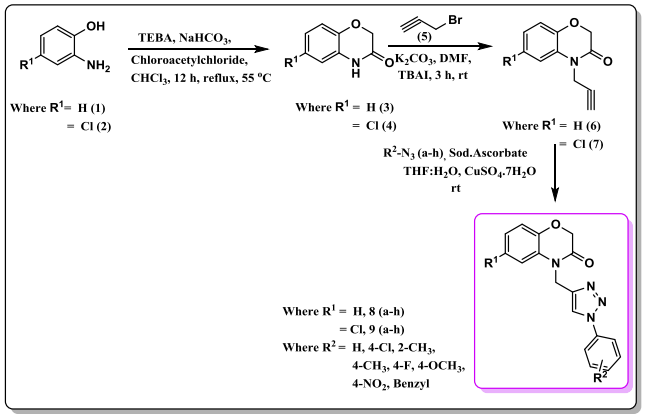


Fig. 1. Structures of 1,4-benzoxazinone-based pharmaceuticals and some bioactive compounds with (1,2,3)-triazole scaffold.



Scheme 1. Synthesis of 4-(1*H*-1,2,3-triazol-4-yl)methyl)-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one derivatives **8a–h** and **9a–h**.

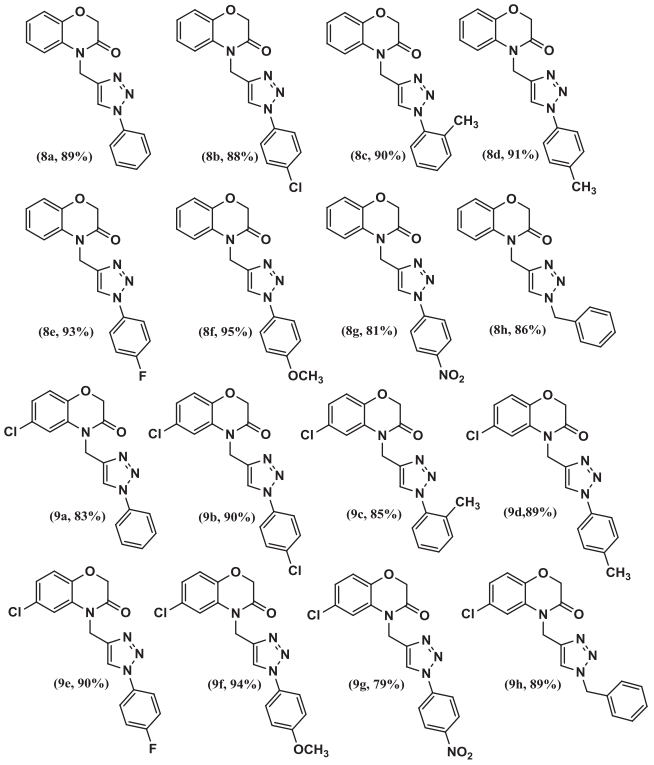


Fig. 3. 1,4-Benzoxazine-1,2,3-triazole hybrids **8a–h** and **9a–h**.

however, the triazole-based drugs are more selective for fungi as compared to mammalian cells which are selective to the azole-based antifungal compounds.[25] 1,2,3-Triazole is a privileged scaffold present in diverse bioactive molecules functioning as anti-cancer (**VII**), anti-fungal (**VIII**),[26] antibacterial,[27,28] anti-allergic,[29] anti-HIV(**IX**),[30,31] anti-tubercular (**X**)[32,33] and anti-inflammatory agents.[34] The importance of (1,2,3)-triazole molecules[35] based on their biological activities are illustrated in Fig. 1.[35]

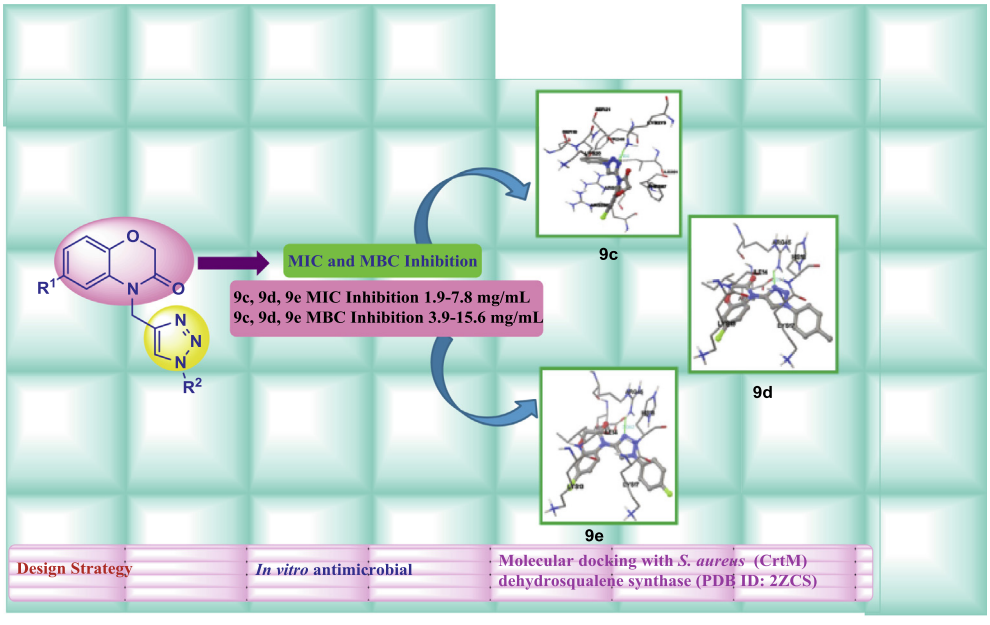


Fig. 2. Design strategy for 4-(1*H*-1,2,3-triazol-4-yl)methyl)-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one hybrids.

In view of biological significance of benzoxazinones and triazoles, it was planned to synthesize benzoxazinone-based triazole containing substituted functional group derivatives which were further screened for *invitro* antimicrobial activities. In this context, the Cu(I) catalyzed click chemistry approach was applied to prepare efficiently various 1,2,3-triazole derivatives. In continuation to our ongoing research activities,[36] to discover and develop new antimicrobial agents, we herein report an efficient method for the synthesis of novel 1,4-benzoxazine-1,2,3-triazole hybrids (**8a**–**h** and **9a**–**h**) with excellent yields.

A series of substituted triazole-functionalized 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one derivatives **8a**–**h** and **9a**–**h** were synthesized in three steps starting from amino phenol using propargyl bromide and substituted azides as intermediates. The first synthetic step involved the acylation of substituted aminophenol (**1** and **2**) with TEBA, $NaHCO_3$ and chloroacetyl chloride in chloroform for about 12 h and "*in situ*" cyclization afforded substituted benzoxazinones **3** and **4**. The key propargyl benzoxazine intermediates (**3** and **4**) was prepared from benzoxazinones (**1**, **2**) by using K_2CO_3 and catalytic amount of TBAI, propargyl bromide (**5**) in DMF solution for about 3 h to afford the corresponding tertiary amines **6** and **7** under room temperature conditions with good yields. Finally, using an one-pot protocol, 1,4-benzoxazin-1,2,3-triazole hybrids **8a**–**h** and **9a**–**h** were obtained by reacting 4-(prop-2-yn-1-yl)-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-ones (**6**, **7**) with various substituted azides (**a**–**h**)[37] via copper (I)-catalyzed 1,3-dipolar cycloaddition (Scheme 1) under room temperature conditions (Figs. 2 and 3).

Table 1
Antimicrobial activity of the synthesized triazolyl benzoxazine hybrids **8a**–**h** and **9a**–**h**.

Test compounds	Minimum inhibitory concentration (μg/mL)							
	Micrococcus luteus MTCC 2470	*Staphylococcus aureus* MTCC 96	*Staphylococcus aureus* MLS-16 MTCC 2940	*Bacillus subtilis* MTCC 121	*Escherichia coli* MTCC 739	*Klebsiella planticola* MTCC 530	*Pseudomonas aeruginosa* MTCC 2453	*Candida albicans* MTCC 3017
8a	–	3.9	–	–	–	3.9	–	–
8b	–	–	–	–	–	–	–	7.8
8c	–	–	3.9	–	3.9	3.9	–	7.8
8d	–	–	3.9	–	3.9	3.9	–	7.8
8e	–	–	–	–	7.8	–	–	3.9
8f	–	–	–	–	7.8	–	–	3.9
8g	–	–	–	–	–	–	–	15.6
8h	–	–	1.9	–	3.9	–	–	–
9a	–	–	–	–	–	–	–	–
9b	–	–	–	–	–	–	–	–
9c	1.9	1.9	1.9	1.9	3.9	3.9	–	7.8
9d	1.9	1.9	1.9	1.9	–	3.9	–	7.8
9e	1.9	3.9	1.9	1.9	3.9	3.9	–	7.8
9f	–	–	1.9	–	–	–	–	7.8
9g	–	–	–	–	–	–	–	7.8
9h	–	–	–	–	–	–	–	7.8
Miconazole (Standard)	–	–	–	–	–	–	–	7.8
Ciprofloxacin (Standard)	0.9	0.9	0.9	0.9	0.9	0.9	0.9	–

Table 2
Minimum bactericidal/fungicidal concentration (MBC/MFC) of the synthesized triazolyl benzoxazine hybrids **8a**–**h** and **9a**–**h**.

Test compounds	Minimum bactericidal/fungicidal concentration (μg/mL)							
	Micrococcus luteus MTCC 2470	*Staphylococcus aureus* MTCC 96	*Staphylococcus aureus* MLS-16 MTCC 2940	*Bacillus subtilis* MTCC 121	*Escherichia coli* MTCC 739	*Klebsiella planticola* MTCC 530	*Pseudomonas aeruginosa* MTCC 2453	*Candida albicans* MTCC 3017
8a	–	7.8	–	–	–	7.8	–	–
8b	–	–	–	–	–	–	–	15.6
8c	–	–	7.8	–	7.8	7.8	–	15.6
8d	–	–	7.8	–	7.8	7.8	–	15.6
8e	–	–	–	–	15.6	–	–	7.8
8f	–	–	–	–	15.6	–	–	7.8
8g	–	–	–	–	–	–	–	31.2
8h	–	–	3.9	–	7.8	–	–	–
9a	–	–	–	–	–	–	–	–
9b	–	–	–	–	–	–	–	–
9c	3.9	3.9	3.9	3.9	7.8	7.8	–	15.6
9d	3.9	3.9	3.9	3.9	–	7.8	–	15.6
9e	3.9	7.8	3.9	3.9	7.8	7.8	–	15.6
9f	–	–	3.9	–	–	–	–	15.6
9g	–	–	–	–	–	–	–	15.6
9h	–	–	–	–	–	–	–	15.6
Miconazole (Standard)	–	–	–	–	–	–	–	7.8
Ciprofloxacin (Standard)	0.9	0.9	0.9	0.9	0.9	0.9	0.9	–

The synthesized hybrids **8a**–**h** and **9a**–**h** were evaluated for their *in vitro* antimicrobial activity against *Micrococcus luteus* MTCC 2470, *Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MLS-16 MTCC 2940, *Bacillus subtilis* MTCC 121, *Escherichia coli* MTCC 739, *Klebsiella planticola* MTCC 530, *Pseudomonas aeruginosa* MTCC 2453 and *Candida albicans* MTCC 3017, and the results to this regard are tabulated in Table 1. Ciprofloxacin and Miconazole were used as standard controls for the bacterial and fungal strains, respectively. The compounds **9c**, **9d** and **9e** exhibited promising and broad spectrum antimicrobial activity against all the tested pathogens except for *Pseudomonas aeruginosa* MTCC 2453 with MIC values ranging from 1.9 to 3.9 μg/mL. Further, the compounds **9c**, **9d** and **9e** exhibited an MIC value of 7.8 μg/mL against *Candida albicans* MTCC 3017. From a structure-activity relationship (SAR) perspective, it was observed that the synthesized compounds **9c**, **9d** and **9e** has *ortho*-methyl, *para*-methyl and 4-fluoro substituents, respectively, attached to the chloro-benzoxazinone scaffold, which exhibited strong electron donating and/or electron withdrawing properties that plausibly may be contributing to the antibacterial and anti-*Candida* activities. While, the compounds **8c** and **8d** exhibited antibacterial activity against *Staphylococcus aureus* MLS-16 MTCC 2940, *Escherichia coli* MTCC 739 and *Klebsiella planticola* MTCC 530 with MIC values of 3.9 μg/mL. Further, these compounds **8c** and **8d** also exhibited antifungal activity against *Candida albicans* MTCC 3017 with MIC value of 7.8 μg/mL. Further, the synthesized compounds **8c** and **8d** have *ortho*-methyl and *para*-methyl substituents attached to the benzoxazinone scaffold which may be exhibiting strong electron donating and/or electron withdrawing properties that may be attributing to the antibacterial and anti-*Candida* activities. In addition, the benzoxazinone derivatives **8b**, **8e**, **8f**, **8g**, **9c**, **9d**, **9e**, **9f**, **9g** and **9h** exhibited promising antifungal activity against *Candida albicans* MTCC 3017 with MIC values ranging between 7.8 and 31.2 μg/mL. Furthermore, all the synthesized compounds were evaluated for the minimum bactericidal concentration (MBC) and the results to this regard are tabulated in Table 2. In this case too, the compounds **9c**, **9d** and **9e** were found to be promising and exhibited broad spectrum antimicrobial activity. Based on these evaluation studies, it was observed that the compounds **9c**, **9d** and **9e** were identified as the most promising leads among all the tested derivatives.

Molecular docking has become a versatile and most important computational method for the prediction of protein-ligand interactions[38] and is an essential and powerful tool for rational drug designing.[39] Over the past several years using high throughput protein purification, protein-ligand complex structures were isolated and were solved using X-ray and NMR spectroscopic methods. Further significant improvements in the computational methods for studying ligand interactions with biological targets have increased in the recent past. In the present study, the three dimensional crystal structure of the C(30) carotenoid dehydrosqualene synthase (CrtM) from *Staphylococcus aureus* complexed with bisphosphonate BPH-700 (PDB ID: 2ZCS) was retrieved from protein databank. All the lead compounds were docked using Autodock 4.2 software against the promising antimicrobial target, dehydrosqualene synthase. For each ligand, the docking score was calculated in terms of kcal/mol. The docking score and H-bond interactions were determined for the lead compounds identified in this study. Among all the screened compounds, **8a**, **8c**, **8d**, **8h**, **9c**, **9f** were found to be interacting with Lys273 and **9f** interacted with Tyr248. Compound **9f** exhibited highest binding energy of −7.04 kcal/mol, showing H-bond interactions with Tyr248 and Lys273, while compound **8h** showed H-bond interactions with Val268 and Lys273. It was observed that all the screened compounds showed good binding energy and H-bond interactions with dehydrosqualene synthase. The docking interaction analysis is illustrated in Fig. 4. The molecular docking studies revealed that the compounds **8a**–**h** and **9a**–**h** exhibited strong H-bond interactions and high binding energies with dehydrosqualene synthase. The compounds with their docking scores and the hydrogen bond interactions of these ligands with amino acid residues in the active site of target protein are provided in Table 3.

In conclusion, we have synthesized a series of triazolyl benzoxazine derivatives **8a**–**h** and **9a**–**h** using simple, three-step procedure with excellent yields. The entire series of synthesized derivatives were screened for antimicrobial properties. Further, docking studies were also performed on these promising derivatives to validate our wet-lab antimicrobial studies. In general, the majority of target compounds displayed moderate to promising activity against the tested pathogenic strains. Based on the antimicrobial studies, the compounds **9c**, **9d** and **9e** showed promising

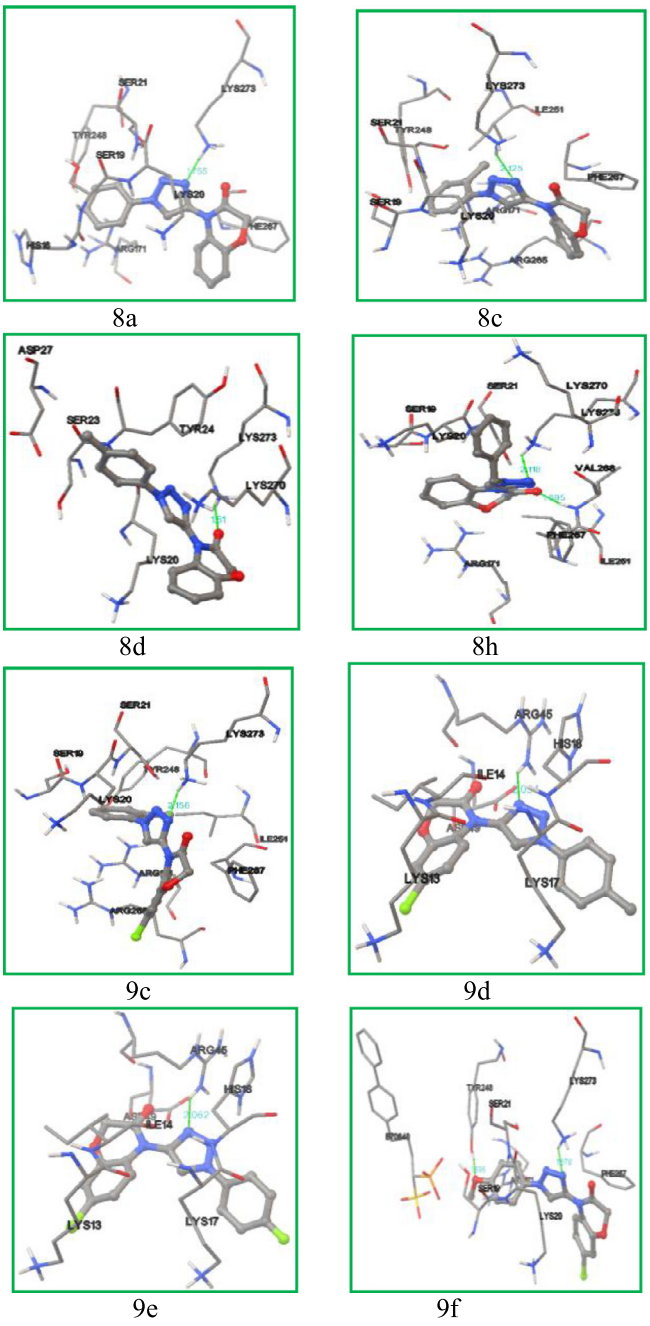


Fig. 4. Docking of all the lead compounds with active site of *Staphylococcus aureus* (CrtM) dehydrosqualene synthase (PDB ID: 2ZCS; deposited on 2007-11-11 and it was released on 2008-03-11).

Table 3
Molecular docking studies of all lead compounds with the active site of *Staphylococcus aureus* (CrtM) dehydrosqualene synthase (PDB ID: 2ZCS).

Test compounds	Interacting amino acids	Binding energy, ΔG (kcal/Mol)	Dissociation constant (kI) (μM)
8a	Lys273	−6.61	14.23
8c	Lys273	−6.62	14.15
8d	Lys273	−6.81	10.26
8h	Val268, Lys273	−6.59	14.84
9c	Lys273	−6.82	10.06
9d	Arg45	−6.86	9.43
9e	Arg45	−6.74	11.42
9f	Tyr248, Lys273	−7.04	6.91

antibacterial and antifungal activities against the pathogenic strains. Docking studies showed that the compounds **8h** and **9f** showed H-bond interactions with high binding energies, while compounds **9c**, **9d** and **9e** showed good H-bond interactions with moderate binding energies. In light of the above, these studies provide insights to develop new drug leads to target the virulence factor, dehydrosqualene synthase (CrtM) of *S. aureus*.

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A. Supplementary data

Supplementary data (experimental section and copies of the ^{1}H NMR, ^{13}C NMR HRMS and IR spectra for some of the important compounds) associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmcl.2017.10.061.

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